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14. ABSTRACT Pain is a common and distressing symptom that impacts the quality of life of many patients with neurofibromatosis. The pain is often due to the formation of a neuroma. To understand better how neuromas cause pain and what treatments may be provided, we have attempted to develop an animal model of a painful neuroma. We have completed the first of the three specific aims in this research project. The tibial neuroma transposition (TNT) model has been confirmed as a model of neuropathic pain. The TNT model has been established as reliable and valid. In the TNT model, the neuroma test-site mechanosensitivity is dependent on neural input from the tibial neuroma. In the TNT model, hindpaw mechanical hyperalgesia is independent of input from the tibial neuroma. We will now move on with work related to developing methods to prevent painful neuroma formation.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	12
Reportable Outcomes.....	13
Conclusion.....	14
References.....	
Appendices.....	15

INTRODUCTION

A percentage of NF1 patients may experience an increase in pain after surgical removal of a neurofibroma. This pain is due to the formation of a painful neuroma, a jumbled mass of nerve fibers and connective tissues, at the cut end of the nerve. Palpating the tissue overlying a neuroma evokes paresthesias/dysaesthesias in the distribution of the injured nerve. Surgical resection of the neuroma may provide relief, but the pain often recurs following the inevitable evolution of a new neuroma at the nerve end. Previous animal models of neuropathic pain have focused on the mechanical hyperalgesia and allodynia that develops at a location distant from the site of injury and not on the pain from direct stimulation of the neuroma. We describe a new animal model of neuroma pain, the tibial neuroma transposition (TNT) model, in which the neuroma is located in a position that is accessible to mechanical testing and outside of the innervation territory of the injured nerve. This allows testing of pain in response to mechanical stimulation of the neuroma (which we call neuroma tenderness) independent of pain due to mechanical hyperalgesia. Mechanical stimulation of the neuroma produced a profound withdrawal behavior that could be distinguished from the hyperalgesia that developed on the hindpaw. The ultimate objective of this research is to prevent reformation of a painful neuroma by using suicide transport of neuronal toxins.

BODY

We will present a summary of our efforts that represent 1, research based directly on the specific aims of the grant and 2, outgrowth research to improve methodology in this work and increase our understanding of the patho-physiology underlying neuropathic pain.

1) Specific Aim Directed Research

We began our experiments by firmly establishing the TNT model with the addition of sufficient animal numbers to our preliminary work to produce a reliable, statistical and publishable result. We then completed our first specific aim by demonstrating that blocking neural input from the neuroma to the CNS reversed the pain behavior produced by the TNT model.

Establishing the TNT Model

The objective of the tibial neuroma transposition surgery is to produce a neuroma that is located in a position accessible for mechanical testing and outside of the innervation territory of the injured nerve. This allows testing of pain in response to mechanical stimulation of the neuroma (which we call “neuroma tenderness”) independent of pain due to hyperalgesia.

Method

As illustrated in Figure 1 (see also Figure 3A), the posterior tibial nerve was exposed from approximately 8 mm proximal to the calcaneal branch to 1 mm distal to the plantar nerve bifurcation. The integrity of the calcaneal branch was preserved while it was dissected free from the main trunk of the tibial nerve. Just proximal to the plantar bifurcation, the tibial nerve was tightly ligated with 6-0 silk and sharply transected with scissors. Using a blunt glass probe, a subcutaneous tunnel was burrowed from the medial incision site to the lateral aspect of the hind limb. A 1.5 mm diameter plastic tube with a longitudinal slit in one wall was placed in the

tunnel. The needle-bearing end of the suture used to ligate the tibial nerve was passed through the plastic tube and pushed through the skin at a location 8-10 mm superior to the lateral malleolus. The plastic tube was then removed from the tunnel. The suture was gently pulled to advance the tibial nerve stump through the subcutaneous tunnel, until it was flush with the inner surface of the skin of the lateral hind limb. The suture was then cut flush with the skin. The incision was closed with running 6-0 silk sutures. The subsequent neuroma was located in a lateral position that was easily accessible for mechanical testing (Figure 1, top). The suture material could be viewed just below the skin surface and provided a target for mechanical testing.

Surgical Procedures

Sham surgery (S)

The tibial nerve was dissected as described above and left intact. A subcutaneous tunnel was formed as described above. A small piece of connective tissue was ligated and passed through the subcutaneous tunnel in the method described above (Figure 3B). The incision was closed with running 6-0 silk sutures.

Tibial neuroma with no transposition (TNT- nT)

The tibial nerve was dissected, ligated, and transected as described above for the TNT model surgery, but not transposed. A subcutaneous tunnel was formed and connective tissue was ligated and passed through to the lateral hindlimb as described above (Figure 3C). The incision was closed with running 6-0 silk sutures.

Tibial neuroma transposition with simultaneous proximal transection (TNT - sPT)

The TNT model surgery was performed as described above. Once the nerve stump was in place on the lateral aspect of the foot, the tibial nerve was sharply transected with scissors at the proximal entrance of the subcutaneous tunnel (Figure 3D). The incision was closed with running 6-0 silk sutures.

Tibial neuroma transposition with delayed proximal transection (TNT - dPT)

The TNT model surgery was performed as described above. Twelve days after surgery, the animals were re-anesthetized, and the tibial nerve was dissected free. Three millimeters proximal to the tunnel entrance, the nerve was tightly ligated with 6-0 silk and a 2-3 mm segment of the tibial nerve distal to the ligature was removed (Figure 3E). A 6-0 silk suture was then used to anchor adjacent connective tissue to close the entrance to the tunnel. For control animals, the nerve was exposed but not cut. The incision was closed with running 6-0 silk sutures.

Lidocaine Block

Nine weeks following TNT surgery, a cohort of eight rats displaying elevated behavioral response frequencies to mechanical stimulation of the neuroma and plantar mechanical hyperalgesia were randomly assigned to two interventional groups, local lidocaine injection or control lidocaine injection. In pairs, the animals were lightly anesthetized using 2% Isoflurane. One animal received a 100 µl injection of 1% Lidocaine with norepinephrine to the neuroma target site marked by the suture on the tibial nerve. To control for systemic effects of lidocaine, the lidocaine/epinephrine was injected into the subcutaneous tissue overlying the lumbar spine of

the other animal. Ten minutes after awakening from anesthesia, the animals were placed in cages on top of the testing stage as described above. Blinded behavioral testing of the neuroma and hindpaw were performed as described above immediately prior to lidocaine injection and three times after injection (15 min, 60 min, 120 min). Two days later, the animals were crossed-over to the other treatment arm and the behavioral protocol was repeated.

Behavioral Testing

To insure blinding, the experimenters doing the behavioral testing were blinded to the surgery of each animal, and the different surgical groups were tested concurrently. The rats were tested three times preoperatively and several times during the postoperative period. The animals were placed in individual transparent plastic cages on top of an elevated wire mesh stage that allowed access to the plantar surface of the paw. A 2.5 x 20 cm window at the bottom of the sidewalls of the cages permitted application of von Frey filaments to the ankle region. The animals were allowed to acclimate to the testing environment for 20-30 minutes before testing began.

Neuroma Tenderness

The suture tied to the distal end of the tibial nerve or connective tissue was visible through the skin and served as the target for mechanical stimuli. An analogous site served as the target on the contralateral hindlimb. A trial consisted of a train of five applications of a von Frey filament (150 mN for 1-2 s) with an interstimulus interval of 1 s. If the animal responded to any of the five applications, the trial was terminated. A positive response was defined as a slow withdrawal of the hindpaw, or rapid withdrawal with vocalization, licking, or shaking. Each testing session consisted of five trials to each hindlimb with an intertrial interval of about 2 minutes. The Response Frequency was defined as the percent of positive trials (i.e., 100 times the number positive trials divided by five).

In later experiments we implemented a grading system to qualitatively evaluate behavioral responses. Each trial was then assigned a response grade ranging from 0 to 2 based on the animal's response. A grade of 0 indicated that the animal did not respond during a given trial. A grade 1 response represented a slow withdrawal of the paw. A grade 2 response was defined as a brisk withdrawal or shaking, licking, or vocalization. The Withdrawal Score was defined as the sum of response grades for the five trials and ranged from 0 to 10.

Whether the response was specific to mechanical stimuli applied to the target site was evaluated in a small cohort of animals following TNT surgery. Testing was performed as described above, but in addition to the neuroma test-site, stimuli were applied to skin overlying the tibial nerve 3 mm proximal to the neuroma, and the skin of the lateral hindlimb 3 mm and 5 mm inferior to the neuroma.

Hindpaw mechanical hyperalgesia

Mechanical withdrawal threshold to the application of a Von Frey probe to the foot was measured by using the up-down method. An ascending series of Von Frey hairs of logarithmically incremental force (3.2, 5.2, 8.3, 15, 29, 44, 64, 94, and 160 mN) were applied to

sites in the middle (tibial nerve distribution) and lateral (sural nerve distribution) aspect of the plantar surface of the left hindpaw (Figure 2). Each Von Frey hair was applied to the test area for about 2-3 s, with a 1-2 min interval between stimuli. A trial began with the application of the 15 mN Von Frey probe to the left and right hindpaws of each animal. A positive response was defined as a rapid withdrawal and/or licking of the paw immediately upon application of the stimulus. Whenever a positive response to a stimulus occurred, the next smaller Von Frey hair was applied, and whenever a negative response occurred, the next higher force was applied. The testing continued for five more stimuli after the first change in response occurred, and the pattern of responses was converted to a 50% von Frey threshold. If the animal showed no response to the highest von Frey hair (160 mN), a von Frey threshold of 260 mN, corresponding to the next log increment in potential von Frey probes, was assigned to the threshold.

Experimental Design

Three separate groups of animals were included in the experiments described in this study.

Experiment Group One

The aim of the initial experiment group was to demonstrate that the TNT model surgery led to the formation of a neuroma associated with a behavioral response that could be evoked by applying mechanical stimuli to the skin overlying the neuroma. The TNT surgery and the three different control procedures performed in experiment group one are illustrated in Figure 3. The TNT model surgery was performed in eight animals (Figure 3A). The three different control procedures were aimed at confirming that the pain behavior in response to palpating the ligature site was due to the neuroma formation. These control procedures were performed concurrently and were therefore also useful in blinding the experimenters. For eight animals, the TNT model surgery was performed, but the tibial nerve was simultaneously transected proximal to the tunnel (TNT-sPT, Fig. 3D). For eight additional animals, the tibial nerve was ligated and cut but not transposed to the lateral location (TN-nT, Fig. 3C). Finally, the tibial nerve was exposed but not cut in eight sham animals (S, Fig. 3B). For all animals, a tunnel was created and a suture was placed under the skin. Behavioral testing for mechanical sensitivity at the neuroma test site was performed in all of the animals. In animals with the TNT, mapping of the behavioral response following application of stimuli at sites distant from the neuroma was also performed.

Experiment Group Two

The aim of the second experimental group was to investigate the effects of the TNT model on paw withdrawal thresholds to mechanical stimuli applied to the plantar surface of the hindpaw.

In addition to TNT surgery (N=8), two control groups were included in this experiment group. Eight animals underwent a sham procedure ("S", Fig. 3B), and eight animals did not undergo any surgical procedures ("C", Fig. 3F). All animals were tested for mechanical hyperalgesia in the hindpaw, as well as mechanical sensitivity at the lateral ankle (i.e., the neuroma test-site). At the conclusion of eight weeks of behavioral testing, the eight animals that had received the TNT model surgery were selected for the lidocaine experimental protocol described above.

Experiment Group Three

The aim of the third experiment group was to determine if the behaviors provoked by applying mechanical stimuli to neuroma test site or plantar hindpaw depended on the presence of the neuroma at the lateral testing site. Twenty four animals underwent TNT model surgery. Twenty animals that demonstrated robust neuroma tenderness and plantar hyperalgesia were selected and divided into two surgical groups. Ten days after the TNT surgery, 11 animals received a delayed proximal transection of the tibial nerve (TNT-dPT, Fig. 3E). To control for the effects of re-exposing the tibial nerve, the tibial nerve was exposed but left intact in the remaining nine animals. All animals underwent additional behavioral testing of the neuroma test site and lateral aspect of the plantar hindpaw.

Statistical analysis

Since the behavioral scoring methods employed yield discrete prefixed values rather than a continuum, and since the data were not normally distributed because of the ceiling effects of a limited range of von Frey hairs, nonparametric tests were performed. Tests were performed to analyze the variance between testing days (Friedman ANOVA for repeated measurements, followed by Wilcoxon matched pairs when appropriate) and between surgical groups on a given testing day (Kruskal-Wallis ANOVA, followed by Mann-Whitney U-test when appropriate). A p value of <0.05 was considered to be statistically significant. Data are presented as median with 25th and 75th quartiles

Results

Mechanical stimulation of the skin overlying a tibial neuroma produces a behavioral response.

In experiment one, eight animals underwent the tibial neuroma transposition (TNT) surgery in which the tibial nerve was ligated and rotated such that the ligature was positioned at the lateral side of the ankle (Figure 3A). The three control groups in experiment one are illustrated in Figures 3B-D. Mechanical stimulation of the lateral side of the ankle with a von Frey probe normally did not lead to a behavioral response. However, following the TNT surgery, animals developed a vigorous response to von Frey stimulation at the ligature (which could be visualized through the skin). The incidence of response to five trials of mechanical stimulation is plotted as a function of time after the lesion in Figure 4. The response frequency for the TNT group differed significantly from baseline starting on post-operative day 5 and persisting for the duration of the experiment (100 days). The response frequency for the TNT group differed from the three control groups starting on day six and for most of the time points thereafter. There was no consistent difference in response frequency for any of the control groups compared to baseline or each other. These control groups were run concurrently with the TNT model animals to insure blinding of the experimenter. Perhaps the most interesting control group is the TNT- sPT group in which the tibial nerve was ligated and rotated as is done for the TNT model surgery but the tibial nerve was cut simultaneously about 1 cm proximal to the ligature. This group did not display an increased response to mechanical stimulation at the ligature site indicating that the behavior was not due to the surgical manipulations necessary to position the ligature on the lateral side of the foot but rather require that the nervous supply to the ligature site

(and eventual neuroma) was intact.

To confirm that this behavior did not reflect cutaneous hyperalgesia but rather required stimulation of the neuroma, we applied the von Frey probe at four different locations relative to the ligature (Figure 5). Von Frey stimulation to the skin overlying the ligature or along the course of the tibial nerve 3mm proximal to the ligature always evoked a 100% response in all animals. Response frequencies decreased in a distance dependent manner as the probe was applied 3mm (RF=36 + 10%) and 5mm (RF=2 + 2%) inferior to the ligature test-site.

The TNT surgery produces behavioral signs of mechanical hyperalgesia in the hindpaw.

Experiment two served as a randomized, controlled assay for the development of mechanical hyperalgesia in the hindpaw following TNT model surgery. Paw withdrawal thresholds to mechanical stimuli applied to the lateral paw and middle paw are shown in Figure 6. At baseline, there was no difference in withdrawal thresholds at either site amongst the groups. In all groups, the withdrawal thresholds in the middle of the hindpaw (tibial nerve distribution) did not vary significantly from baseline at any time in the postoperative period. The mechanical withdrawal thresholds in the lateral aspect of the hindpaw varied significantly with group and time. Animals in the TNT model group displayed mechanical withdrawal thresholds that were significantly lower than baseline and the naive control group for the duration of the postoperative period (51 days) with the exception of days 17 and 37 when they were only significantly lower than baseline. The TNT model group displayed paw withdrawal thresholds that tended to be lower than those of the sham group on all postoperative days. This difference reached significance on days 23, 44, and 51. For the sham and naive groups, lateral-site paw withdrawal thresholds did not vary significantly from baseline or each other.

Proximal tibial nerve transection reverses the neuroma tenderness produced by the TNT model.

Experiment three evaluated the effect of a delayed, proximal tibial nerve transection on neuroma tenderness. Twenty of the twenty four animals that underwent TNT model surgery displayed behavioral response scores that were significantly greater than baseline six days after surgery. Ten days after the TNT model surgery, 11 of these animals underwent proximal tibial nerve transection. The remaining 9 animals had the tibial nerve exposed (but not cut) and served as the control animals to blind the experimenter.

Following proximal tibial nerve transection, behavioral response scores for stimulation at the neuroma dropped abruptly and were not significantly different from the baseline scores before the TNT surgery. The scores were significantly lower than immediately prior to proximal tibial nerve transection for the entire testing period (Figure 7). In contrast, following tibial nerve exposure in the control group, response scores did not decrease but remained significantly greater than baseline and did not vary significantly from immediately prior to tibial nerve exposure. Behavioral response scores were significantly lower for the proximal tibial nerve transection compared to tibial nerve exposure group on all postoperative test days except day 33. Thus, proximal tibial nerve transection led to a reversal of the neuroma pain behavior.

Mechanical hyperalgesia in the hindpaw produced by the TNT model persists following proximal tibial nerve transection.

Experiment three was also used to assess the effects of proximal tibial nerve transection on mechanical hyperalgesia produced by TNT model surgery. At baseline, paw withdrawal thresholds for the two groups did not differ (Figure 7B). Immediately after TNT model surgery, paw withdrawal thresholds on the lateral side of the foot were significantly lower than baseline for both groups. No difference was evident between the groups. Following proximal tibial nerve exposure or transection, paw withdrawal thresholds remained significantly decreased from baseline for all animals. There was no difference in paw withdrawal threshold between the two groups at any time point. Thus, proximal tibial nerve transection did not lead to a reversal of the hindpaw hyperalgesia.

A total of 40 animals received TNT surgery in the three experimental groups. Thirty six of these animals (90%) displayed a positive behavioral response to mechanical stimulation of the skin overlying the neuroma. Interestingly, later cohorts of animals (experiments 2 and 3) appeared to develop neuroma tenderness more rapidly than the first cohort (experiment 1). The most likely explanation is improvements in surgical and behavioral techniques with experience.

Local lidocaine injection reverses the neuroma tenderness produced by the TNT model, but does not effect hindpaw mechanical hyperalgesia.

Figure 8 illustrates the effects of local lidocaine injection on neuroma tenderness and hindpaw mechanical hyperalgesia compared to the effects of lidocaine injection at a remote site. Eight TNT animals from experiment group two that displayed increased behavioral response frequencies at the neuroma-site and hindpaw mechanical hyperalgesia nine weeks after tibial nerve neuroma model surgery were enrolled in a crossover study. Response frequencies were significantly lowered following local, but not remote lidocaine injection. This was first evident at 15 minutes and lasted for the duration of the experiment (120 minutes). Behavioral response frequencies following remote lidocaine injection did not significantly differ from pre-injection levels.

Paw withdrawal thresholds to mechanical stimuli did not differ from pre-injection levels following remote or local lidocaine injection. Following injection, the withdrawal thresholds of the two groups did not differ with respect to each other.

2) Outgrowth Research

Mechanical Stimulation

In an attempt to improve the reliability and validity of the TNT model we looked at a variety of methods to deliver a mechanical stimulus to the target neuroma. In the current protocol, a von Frey probe is applied to the target. The probe has a tendency to slide on the skin and may not be delivering a consistent stimulus with each application. This leads to variability in the response. Further, a human neuroma responds to a tapping type stimulus more so than to a static mechanical stimulus. To more closely reproduce the human condition, we devised a blunt spring loaded probe stimulator. Our preliminary data in seven animals with the spring loaded probe stimulator suggests it to be more robust and consistent a stimulus versus the von Frey filament (figure 9).

Patho-physiology of Neuropathic Pain

A collaborative research effort has been instituted with Dr. Kazim Sheikh from the Department

of Neurology at Johns Hopkins Hospital. Dr. Sheikh has demonstrated that passive immunization with anti-ganglioside antibodies directly inhibits axon regeneration. We have hypothesized that pre-treating our TNT model animals with these antibodies may prevent the development of a neuroma and subsequent pain related behavior. Funding for this pilot project has been obtained through a competitive grant from the Blaustein Pain Research Fund at The Johns Hopkins University.

We have demonstrated that neuroma related neural activity from the TNT model is involved in neuropathic pain and can be blocked with local application of lidocaine. Several pharmacologic preparations that inhibit neuronal activity have been developed to treat epilepsy. Many of these preparations have also proven to have utility in the treatment of neuropathic pain. We obtained a small grant from the UCB Pharma corporation to investigate if one such drug, levitracetum, will have such an effect on the neuroma in our TNT model. Our preliminary data did not suggest that the drug had an impact on the neuroma pain behavior. This particular project stopped. We have considered various other pharmacologic interventions that we may pursue depending on funding and available resources.

KEY RESEARCH ACCOMPLISHMENTS

- The TNT model has been confirmed as a model of neuropathic pain
- In the TNT model, the neuroma test-site mechanosensitivity is dependent on neural input from the tibial neuroma
- In the TNT model, hindpaw mechanical hyperalgesia is independent of input from the tibial neuroma
- Modification in the behavioral testing methods have improved the ability to utilize the TNT model

REPORTABLE OUTCOMES

A manuscript entitled, The tibial neuroma transposition (TNT) model of neuroma pain and hyperalgesia, is in final preparation for submission to the journal, Pain.

A grant was applied for and funding provided from the Blaustein Fund. The aim of the research is to determine if painful neuroma formation in the TNT model can be prevented by pre-treatment with anti-ganglioside antibodies

CONCLUSION

We have now completed the first of three specific aims and are publishing the results. The pain behavior displayed by the animal results from mechanical stimulation of the neuroma, a phenomenon commonly seen in patients with painful neuroma. The tibial neuroma transposition (TNT) model provides the scientific community an animal model of neuroma pain.

The second specific aim of the research will now be undertaken. We will look at methods of preventing painful neuroma formation including suicide transport of neural toxins (see grant specific aim 2) in addition to exploring other modalities such as anti-ganglioside antibodies.

SUPPORTING DATA

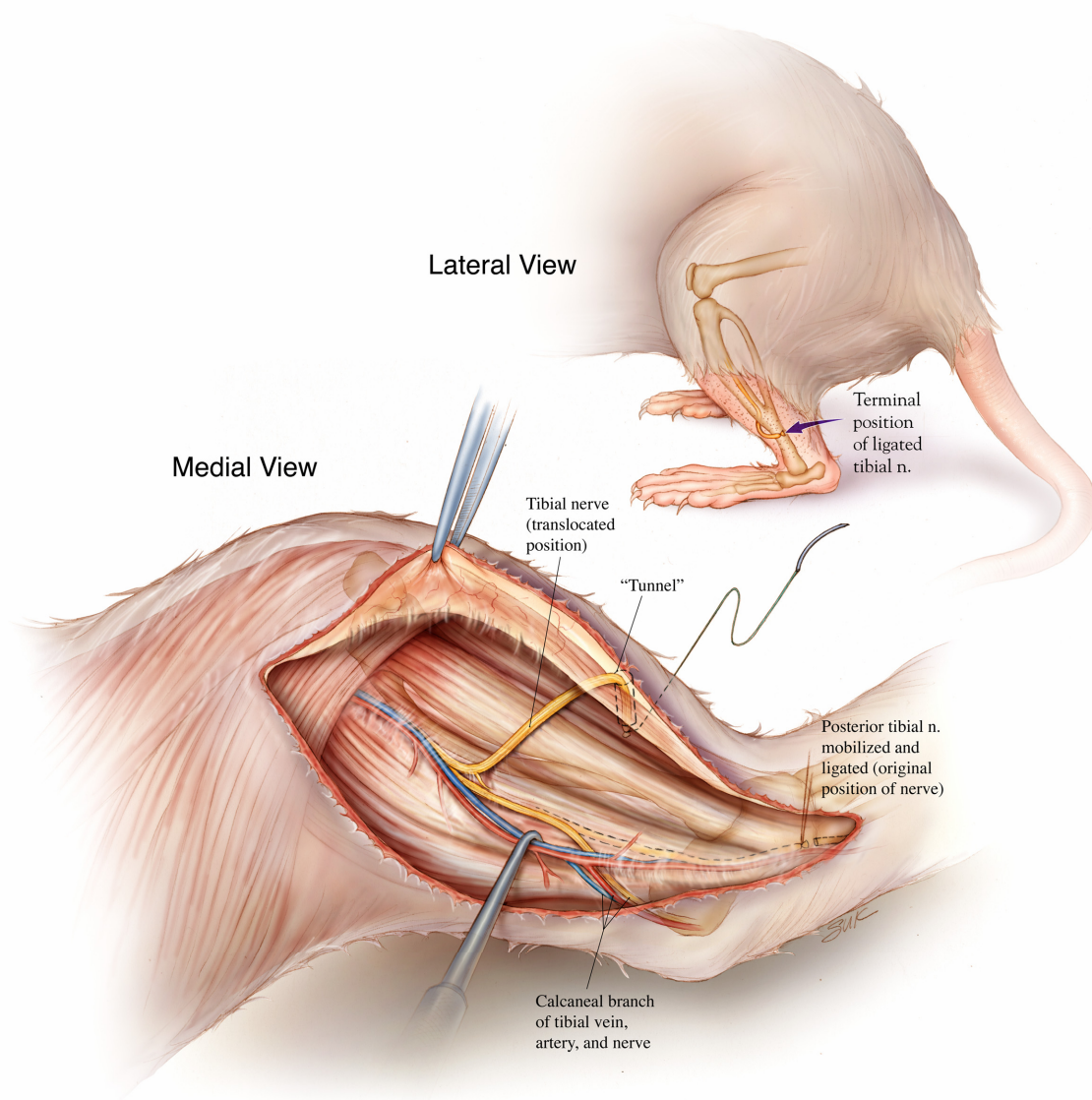


Figure 1: The Tibial Neuroma Transposition (TNT) Model. Schematic depicting TNT model surgery. The distal tibial nerve in the foot is dissected free of adjacent tissue, ligated with a suture, and cut. The needle from the suture is passed through a subcutaneous tunnel to the lateral aspect of the hindlimb where it is pushed through the skin. The nerve is drawn into the tunnel until the ligature is adjacent to the skin. The suture is cut, and the incision closed.

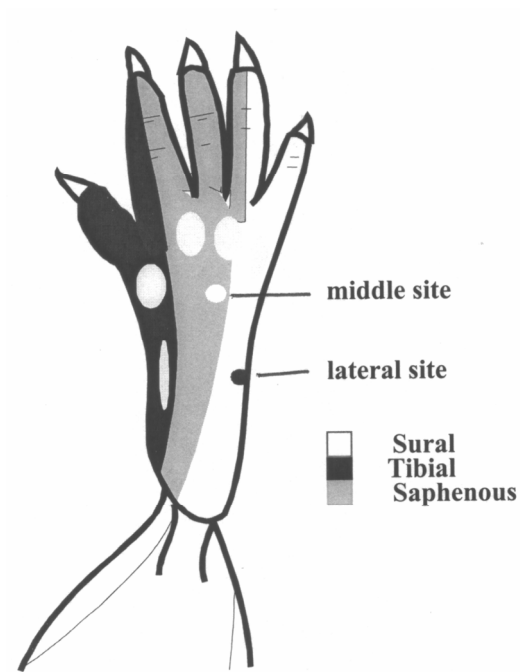
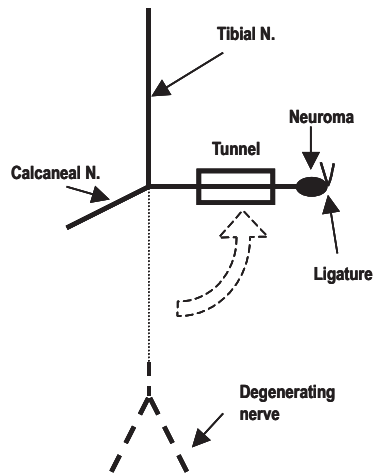
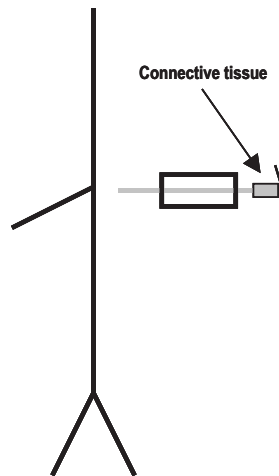


Figure 2: Mechanical hyperalgesia testing sites. To test for mechanical hyperalgesia, von Frey probes were applied to lateral (sural distribution) or middle (tibial distribution) sites on the plantar surface of the paw.

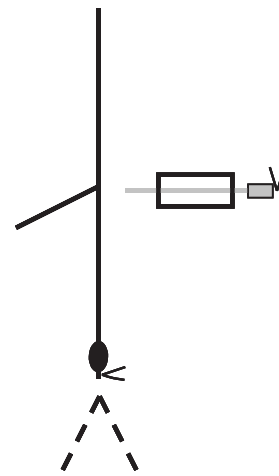
A. Tibial Neuroma Transposition (TNT)



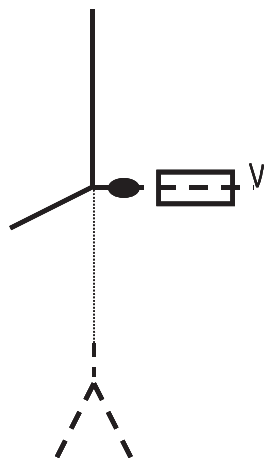
B. Sham (S)



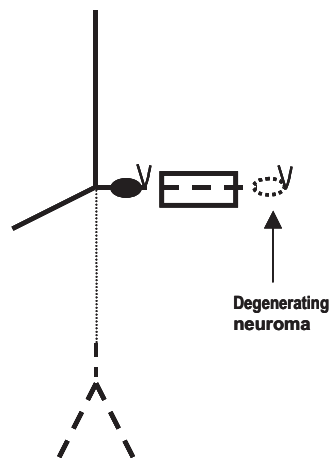
C. Tibial Neuroma, no transposition (TN-nT)



D. TNT with simultaneous proximal transection (TNT-sPT)



E. TNT with delayed proximal transection (TNT-dPT)



F. Control, no surgical procedures (C)

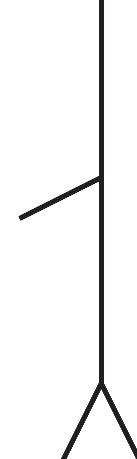


Figure 3: Schematic of the different surgical groups.

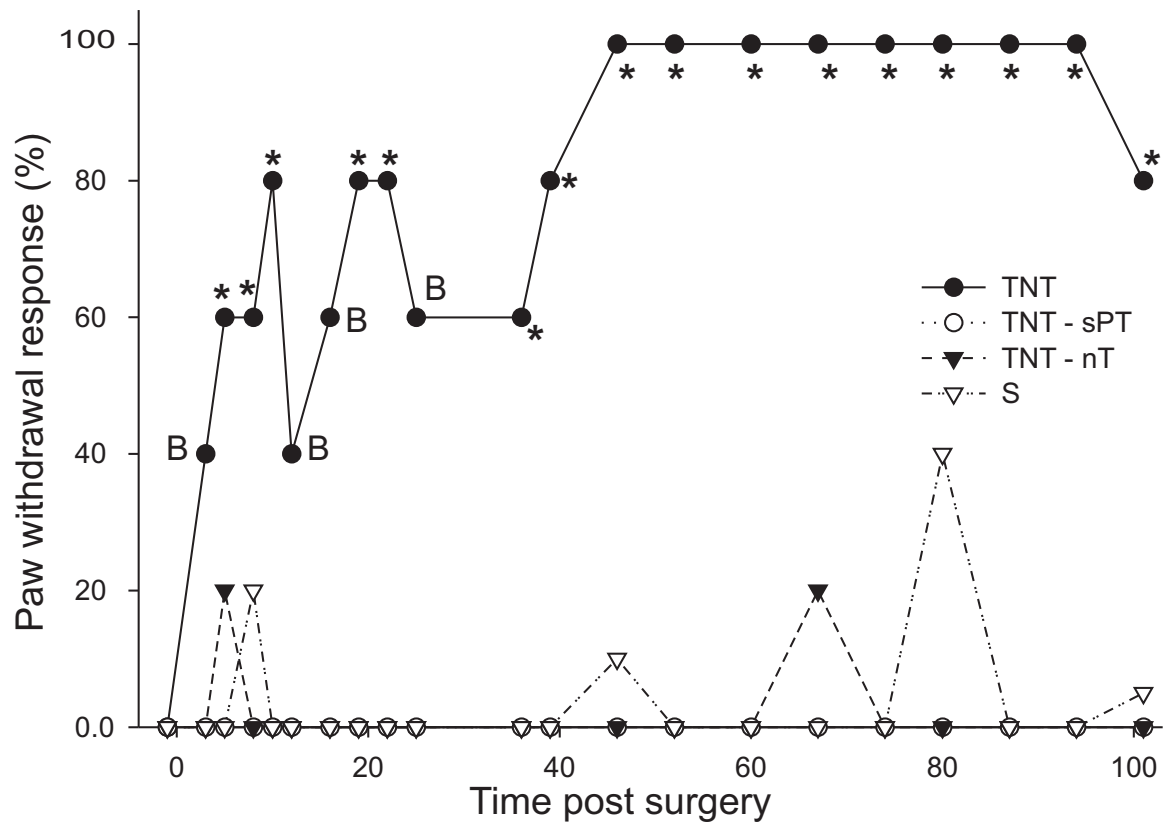


Figure 4: TNT model produces neuroma tenderness. Following TNT surgery, animals displayed an increased frequency of response to application of a 150 mN von Frey probe to the ligature site. The median behavioral response frequency for the TNT group differed significantly from baseline starting on post-operative day 5 (B = $p < 0.05$). The TNT group differed significantly from the three control groups starting on day 7 (* = $p < 0.05$ with respect to baseline and with respect to other groups). The control groups did not differ significantly from baseline or each other. Schematics of the surgeries performed in each of the groups are shown in Fig. 3.

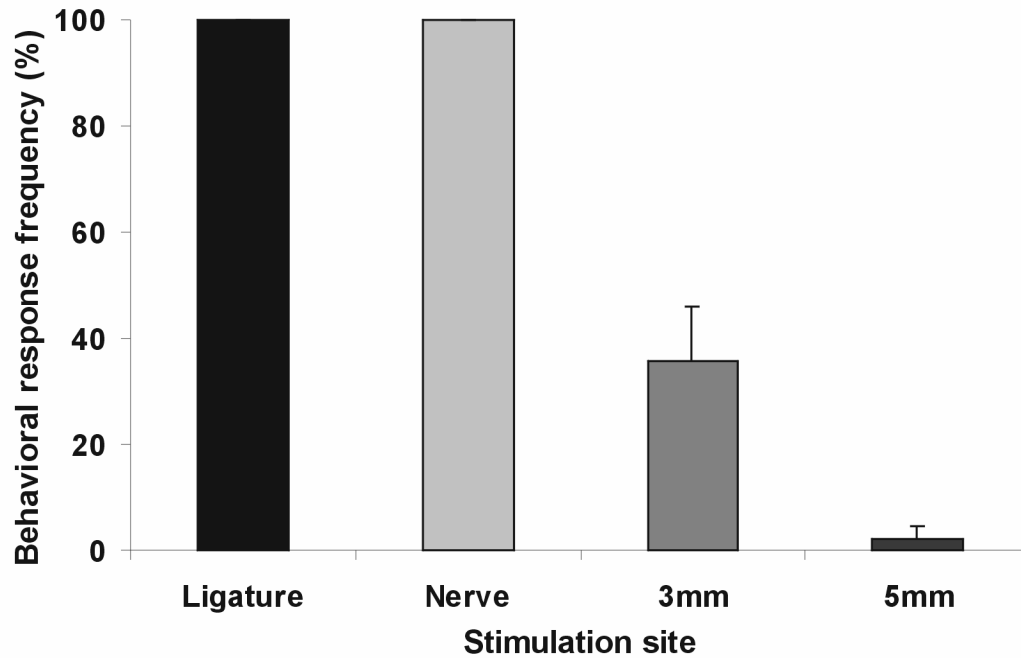


Figure 5: Focal region of neuroma tenderness in TNT model. The behavioral response frequency to application of a 150 mN von Frey probe was measured at four sites on the lateral hindlimb: the ligature site, 3 mm proximal to the ligature (on the tibial nerve), 3 mm inferior to the ligature, and 5mm inferior to the ligature (n=9).

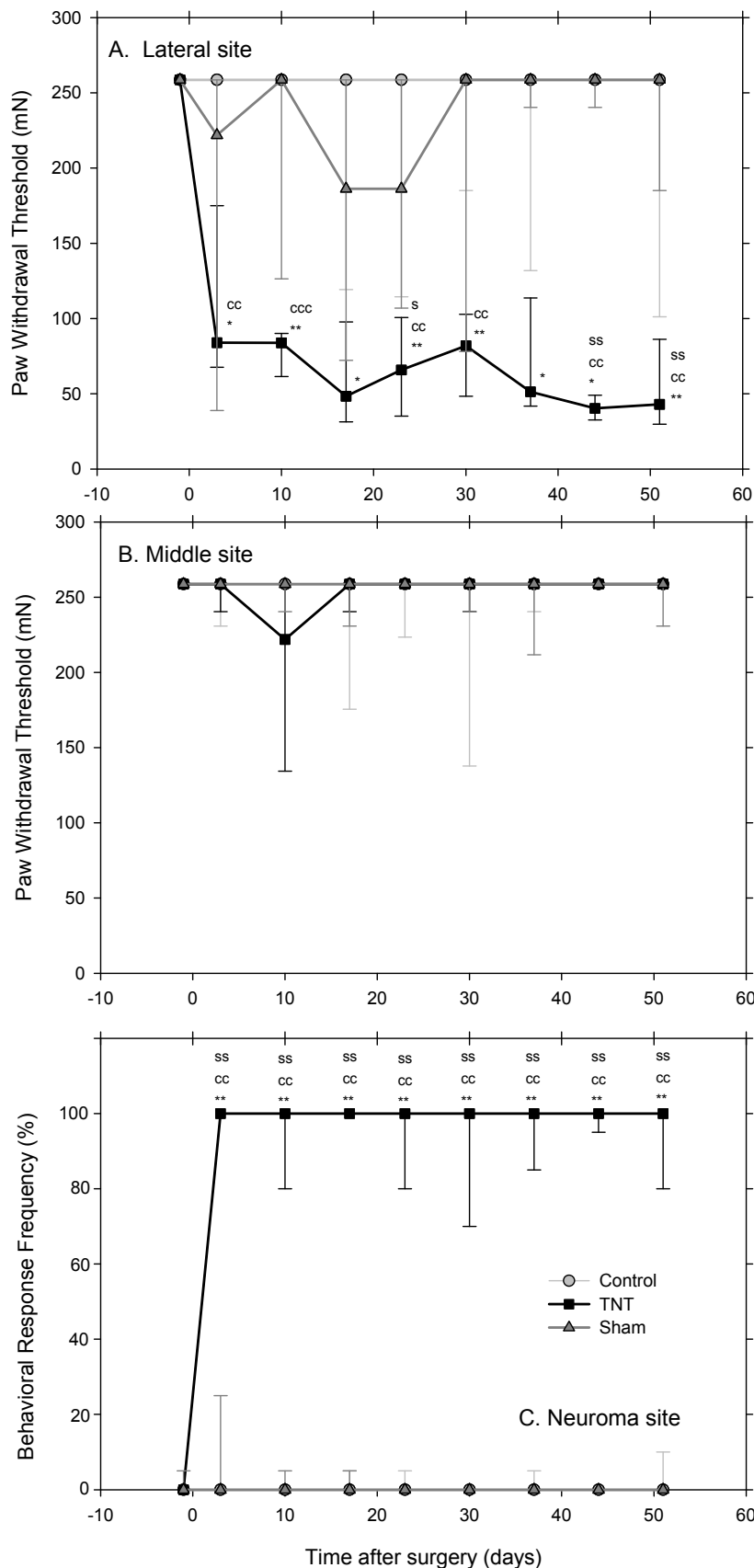


Figure 6: TNT model produces mechanical hyperalgesia. Paw withdrawal thresholds to von Frey stimuli applied to the lateral (A) and middle (B) test sites are plotted as a function of time after the surgery. (A) At the lateral test site, animals in the TNT model group (filled square, $n=8$) displayed mechanical withdrawal thresholds that were significantly lower than baseline (* = $p \leq 0.05$, ** = $p \leq 0.01$), the non-operated control (circle, $n=8$, ^c = $p \leq 0.05$, ^{cc} = $p \leq 0.01$), and the sham group (triangle, $n=8$, ^s = $p \leq 0.05$, ^{ss} = $p \leq 0.01$). There was no difference in withdrawal thresholds at either site amongst the groups at baseline. (B) In all groups, the withdrawal thresholds in the middle of the hindpaw (tibial nerve distribution) did not vary significantly from baseline at any point in the postoperative period. (C) Tenderness over the lateral ankle developed in all animals following TNT model surgery, but not in the sham or control animals. The response frequencies for the TNT model group were significantly elevated compared to non-operated control group (^{cc} = $p \leq 0.01$), the sham group (^{ss} = $p \leq 0.01$), and baseline (** = $p \leq 0.01$).

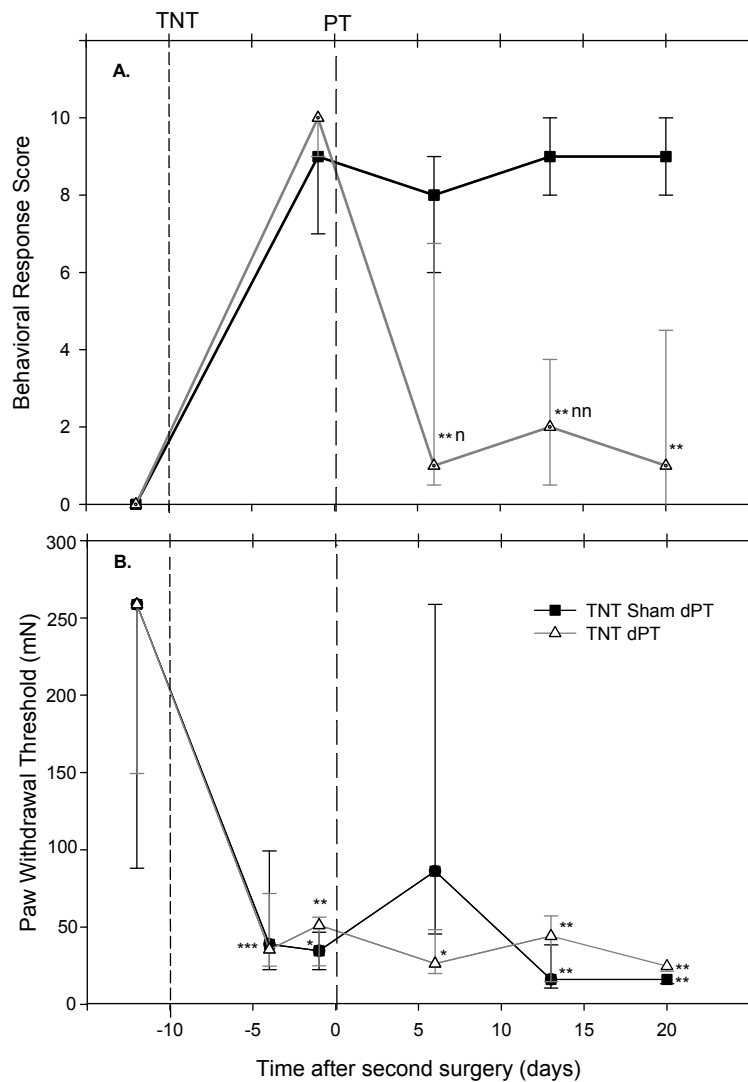


Figure 7: Proximal tibial nerve transection reverses neuroma tenderness

The TNT model surgery was done on all animals. Ten days later, the tibial nerve was exposed 1 cm proximal to the ligature site in animals that had developed robust pain behaviors (n=20). The nerve was transected in 11 of the animals (TNT-dPT) and left alone in the others (TNT sham dPT). (A) Neuroma tenderness is reversed by proximal tibial nerve transection. The TNT surgery produced behavioral response scores that were significantly greater than baseline for both groups. The delayed proximal transection

(dPT), but not sham, resulted in a significant decrease in the behavioral response scores to a level that was not significantly different from baseline. The behavioral response scores remained significantly lower than immediately following TNT model (** = $p \leq 0.01$) for the entire testing period. Compared to sham dPT group, the dPT group demonstrated behavioral response scores that were significantly lower on all postoperative test days except day 33 ($^n = p \leq 0.05$, $^{nn} = p \leq 0.01$). (B) Paw hyperalgesia is not changed by proximal tibial nerve transection. At baseline, paw withdrawal thresholds for the two groups did not differ. Immediately after the TNT surgery, paw withdrawal thresholds were significantly lower than baseline for both groups. No difference was evident between the groups. Following dPT or sham dPT, paw withdrawal thresholds remained significantly decreased from baseline and did not differ from post-TNT model levels (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$). There was no difference in paw withdrawal threshold between the two groups at any time point.

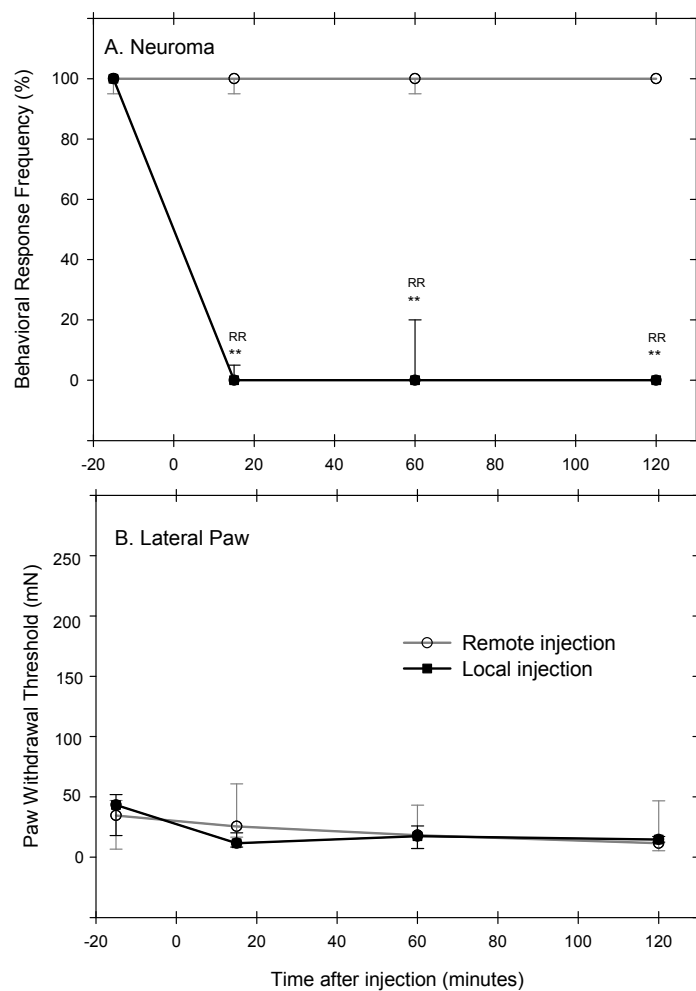


Figure 8: Lidocaine injection at the site of the neuroma reverses neuroma tenderness.

Nine weeks after TNT surgery, lidocaine (1%, 100 μ l) was injected at the site of the neuroma or at a remote site. The local lidocaine injection resulted in a significant decrease in the response frequency to mechanical stimulation of the neuroma (** = $p \leq 0.01$). Injection of lidocaine to a remote site did not affect the response frequency. The response frequencies were significantly lower following injection for the local versus remote injection group for the entire 120 minute test period ($^{RR} = p \leq 0.01$). (B) Lidocaine injection, local or remote, did not alter paw withdrawal thresholds to mechanical stimulation of the plantar hindpaw at any time point following injection.

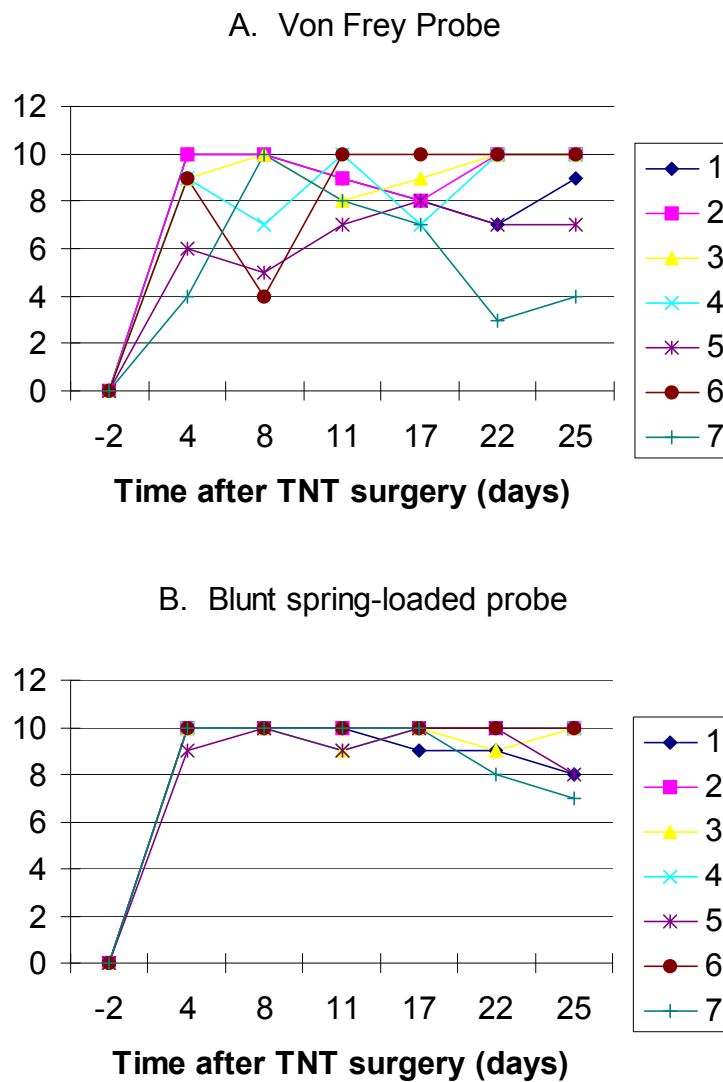


Figure 9. Paw withdrawal scores are plotted as a function of time after TNT surgery for 7 different animals tested with the von Frey probe (A) or the blunt spring-loaded probe (B). The variability in response was greatly reduced with the spring-loaded probe.